Iron metabolism in patients with rheumatoid arthritis

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Research

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Abstract

Background. The most common haematological manifestation in RA is anaemia (30-60%). The aim of the study was the assessment of the prevalence of iron deficiency in RA patients using standard parameters and new biomarkers.

Material and methods. The study was conducted on 62 RA patients, aged 52±15, treated at the Department of Internal Medicine of the 4th Military Teaching Hospital in Wrocław between 2016 and 2017. The control group comprised 58 healthy individuals, aged 56±9. The following tests were carried out: DAS-28, complete blood count, creatinine, uric acid, AspAT, ALAT, GGT, bilirubin, TSH, lipid profile, iron, TIBC, transferrin saturation, ferritin, soluble transferrin receptor, hepcidin, and IL-6.

Results. A higher percentage of RA patients compared with the control group had TSAT values below 20%, ferritin levels below the reference range, soluble transferrin receptor levels above 1.59mg/l and hepcidin levels below 14.5 ng/ml. 60% of RA patients had iron deficiency. The correlations between reduced ferritin levels and younger age, female gender, lower GGT levels and increased platelet counts was shown. The correlations were found between iron deficiency and younger age, female gender, reduced haemoglobin levels, increased platelet counts, increased GFR, reduced GGT levels, lower disease activity and less frequent use of sulfasalazine

Conclusions. Iron deficiency is common in RA patients with high disease activity. RA patients had lower transferrin saturation, lower ferritin and hepcidin levels and higher serum sTfR levels. The increased DAS-28 scores and reduced haemoglobin levels were the two strongest determinants of iron deficiency in RA patients.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by the involvement of numerous joints and by systemic symptoms, which leads to disability and premature death [1]. The most common haematological manifestation in rheumatoid arthritis is anaemia, which affects 30–60% of RA patients and significantly complicates the clinical course of the disease. Anaemia of chronic disease, which is associated with systemic inflammation, is considered the most common type of anaemia in RA patients (> 60%) [2]. However, anaemia may also be associated with iron, vitamin B_{12} or folic acid deficiency. More than one type of anaemia may be simultaneously present in one patient [3].

Iron deficiency may be absolute or functional. It may be present in anaemic patients, but it may also occur in patients with normal haemoglobin levels. Absolute iron deficiency occurs when body iron stores are depleted. It usually results from insufficient iron intake in the diet, impaired absorption of iron from the gastrointestinal tract or its loss as a result of bleeding (usually from the gastrointestinal tract, less often from the urinary or genital tract) [4, 5, 6]. Functional iron deficiency is caused by insufficient availability of iron (which is trapped in macrophages) for cellular processes [4, 5, 6]. This can be observed during inflammation. It was found that both anaemia and iron deficiency (without anaemia) in inflammatory diseases other than RA (e.g. heart failure) may be associated with reduced exercise tolerance and a worse prognosis [6]. Moreover, it was found that there is a causal link between iron deficiency and reduced work capacity [7].

Similarly to functional iron deficiency, anaemia of chronic disease has a multifactorial pathogenesis and is associated with the stimulation of the immune system. During inflammation, activation of immune-competent cells and increased secretion of proinflammatory cytokines impair erythropoiesis (partly because proinflammatory cytokines cause resistance to erythropoietin) and disrupt iron metabolism (partly because proinflammatory cytokines cause iron to be captured by active macrophages and thus it is not available for cells that metabolise iron) [4, 8, 9, 10]. Interleukin 6 (IL-6), which is overexpressed during inflammation and stimulates the production of hepcidin by hepatocytes, plays a key role in these processes [4, 8, 9, 11]. Hepcidin, which binds and internalises ferroportin (protein which transports iron through cell membranes to the outside of the cell), inhibits the absorption of iron from the gastrointestinal tract and inhibits the release of iron stored by macrophages in the reticuloendothelial system [4, 6, 12, 13].

There is evidence that low haemoglobin levels in RA patients are significantly associated with disability, activity and duration of the disease as well as damage to joints and joint pain [14]. Treatment of anaemia in RA patients includes iron supplementation, blood transfusions and the use of erythropoiesis-stimulating agents. Importantly, the treatment of the underlying inflammatory condition itself may lead to an increase in haemoglobin levels [15]. Biological treatments used in RA patients, e.g. infliximab (anti-TNF-α antibody), tocilizumab (anti-IL-6 receptor antibody) and anakinra (anti-IL-1 antibody), not only effectively inhibit the progression of joint involvement, but may also prevent anaemia [16].

The Aim of The Study

The main aim: assessment of the prevalence of iron deficiency in RA patients using standard parameters (ferritin, transferrin saturation) and new biomarkers (soluble transferrin receptor, hepcidin) measured in the peripheral blood of patients with and without anaemia.

Specific objectives:

1) comparison of iron metabolism between RA patients and healthy controls using the aforementioned parameters;
2) analysis of correlations between iron metabolism parameters and haemoglobin levels as well as red blood cell parameters in RA patients;
3) analysis of correlations between iron deficiency and the clinical condition of RA patients, especially disease activity, inflammation parameters, comorbidities and treatment used;

Material And Methods

Inclusion criteria:

a) age: ≥18;

b) RA diagnosed according to the ACR criteria;

c) time from RA diagnosis ≥6 months;

d) active RA (DAS-28>5.1);

e) informed written consent to participate in the study.

Exclusion criteria:

a) treatment with iron, erythropoietin analogues or blood transfusions during the previous 12 months.

A one-off clinical and laboratory assessment of each RA patient was performed. The following tests and examinations were carried out:

a) physical examination, anthropometric measurements and medical history (history of RA, comorbidities and medication taken);

b) Disease Activity Score of 28 joints (DAS-28) [17] – the system is commonly used in the daily rheumatological assessment of RA patients and helps make decisions about modification of treatment. It involves determination of the number of tender (TEN28) and swollen (SW28) joints out of 28 peripheral joints (including 10 proximal interphalangeal joints, 10 metacarpophalangeal joints, 2 wrist joints, 2 elbow joints, 2 shoulder joints and 2 knee joints), assessment of general health status by a patient using a 100-mm visual analogue scale (VAS) as well as C-reactive protein (CRP) levels. DAS-28 is used both to determine RA activity and to assess clinical improvement parameters. The DAS-28 score is calculated according to the following formula [18]:

$$DAS\text{-}28 = 0.56 \times \sqrt{(TEN28)} + 0.28 \times \sqrt{(SW28)} + 0.70 \times \ln(ESR) + 0.014 \times (VAS)$$

RA activity is defined as follows:

DAS-28 >5.1 - high disease activity,
DAS-28 ≤5.1 - >3.2 - moderate disease activity,
DAS-28 ≤3.2 - ≥2.6 - low disease activity,
DAS-28 < 2.6 - remission;

c) laboratory tests to assess the severity of inflammation (ESR, CRP) (standard laboratory methods);

d) laboratory tests to determine the presence/severity of comorbidities (complete blood count, creatinine, uric acid, AspAT, ALAT, GGT, bilirubin, TSH, lipid profile);

e) laboratory tests to assess iron metabolism: iron, TIBC, transferrin saturation, ferritin, soluble transferrin receptor, hepcidin (soluble transferrin receptor levels determined by immunonephelometry and hepcidin levels determined using ELISA);

f) serum level of IL-6, a proinflammatory cytokine.

Iron deficiency was defined as serum ferritin < 100 µg/L or serum ferritin between 100 µg/L and 299 µg/L with TSAT < 20%. Anaemia was defined as haemoglobin levels below 12 g/dL for women and below 13 g/dL for men [19].

The study was approved by the Bioethics Committee at the Military Institute of Medicine in Warsaw (No. 34/WIM/2015).

Continuous variables with normal distribution were presented as a mean and standard deviation, whereas variables with skewed distribution were presented as a median with upper and lower quartiles. The distribution of continuous variables was tested using the Kolmogorov–Smirnov test. The significance of differences for continuous variables with normal distribution was tested using a Student's t-test for unpaired (independent) samples. The significance of differences for continuous variables with skewed distribution was tested using the Mann-Witney test. The significance of differences for categorical variables was tested using the chi-square test. Correlations between continuous variables with normal distribution were tested using Pearson correlation coefficients, whereas correlations between continuous variables with skewed distribution were tested using Spearman's rank correlation coefficients.

Correlations between continuous variables describing iron metabolism (serum ferritin level, TSAT and serum levels of hepcidin and soluble transferrin receptor) as well as haemoglobin levels and clinical and laboratory parameters were analysed using single-factor and multi-factor linear models. Iron deficiency was a dependent variable, whereas other clinical and laboratory parameters were independent variables. Explanatory variables (clinical and laboratory parameters) which were statistically significantly correlated with the prevalence of iron deficiency in single-factor models were included in logistic multi-factor models. In all analyses, $p < 0.05$ denoted statistical significance.

Results

The study was conducted on 62 RA patients aged 52 ± 15 treated at the Department of Internal Medicine of the 4th Military Teaching Hospital in Wrocław between 2016 and 2017. The control group comprised 58 healthy individuals aged 56 ± 9. A majority of RA patients were women (84%). Mean BMI was higher in healthy controls than RA patients. RA patients had the following comorbidities: hypertension (32%), diabetes (6%), asthma (6%), ischaemic heart disease (5%), atrial fibrillation (2%). Both groups were compared in terms of selected laboratory parameters and the following was found in RA patients: lower triglyceride levels, lower uric acid levels, higher CRP levels, higher platelet counts and higher GFR (Table 1).

Table 1
Clinical characteristics, basic laboratory parameters, haematological parameters and iron metabolism parameters in RA patients and healthy controls

Variable	RA patients, n = 62	Control group, n = 58	p
Age [years]	52 ± 15	56 ± 9	0.07
Sex, male, n (%)	10 (16)	35 (60)	< 0.001
BMI - body mass index [kg/m^2]	25.5 ± 4.6	27.3 ± 4.5	< 0.05
Hypertension, n (%)	20 (32)	0	-
Diabetes, n (%)	4 (6)	0	-
Atrial fibrillation, n (%)	1 (2)	0	-
Ischaemic heart disease, n (%)	3 (5)	0	-
Asthma, n (%)	4 (6)	0	-
Triglycerides [mg/dL]	97 ± 40	184 ± 72	< 0.0001
GFR - glomerular filtration rate [mL/min/1.73 m^2]	95 ± 19	87 ± 23	< 0.05
Uric acid [mg/dL]	4.8 ± 1.3	5.7 ± 1.1	< 0.001
CRP - C-reactive protein [mg/L]	3.5 (1.3–17.1)	0.8 (0.5–1.3)	< 0.0001
Platelet count [G/L]	294 ± 90	246 ± 67	< 0.01
Haemoglobin [g/dL]	13.3 ± 1.3	14.3 ± 1.2	< 0.001
Anaemia$^{\$}$, n (%)	11 (18)	3 (5)	< 0.05
RBC [T/L]	4.5 ± 0.4	4.8 ± 0.4	< 0.001
Haematocrit [%]	40.4 ± 5.7	42.2 ± 3.2	< 0.001
MCV [fL]	90.7 ± 5.9	88.4 ± 3.9	< 0.05
MCH < 26 pg (f), < 27 pg (m), n (%)	4 (6)	0	< 0.05
MCHC [g/dL]	32.3 ± 1.2	33.9 ± 1.1	< 0.001
RDW [%]	14.5 ± 1.3	12.9 ± 0.6	< 0.001
Vitamin B_{12} < 200 pg/mL, n (%)	2 (3)	-	-
Folic acid < 3 ng/mL, n (%)	1 (2)	-	-
TSAT < 20%, n (%)	26 (43)	3 (5)	< 0.001
Ferritin < 30 µg/L, n (%)	9 (15)	4 (7)	0.17
Hepcidin < 14.5 ng/mL, n (%)	32 (56)	1 (2)	< 0.0001
sTfR > 1.59 mg/L, n (%)	16 (26.2)	0	< 0.0001
Iron deficiency$^{¥}$, n (%)	39 (64)	-	-
$ Anaemia: haemoglobin < 12 g/dL for women and < 13 g/dL for men			
¥ Iron deficiency: ferritin < 100 µg/L or ferritin 100–299 µg/L and TSAT < 20%			
RA – rheumatoid arthritis			
RBC - red blood cells - erythrocytes			
MCV - mean corpuscular volume			
MCH - mean corpuscular haemoglobin/mean cell haemoglobin			
MCHC - mean corpuscular haemoglobin concentration			
RDW - red cell distribution width			

Variable	RA patients, n = 62	Control group, n = 58	p
PHRC - percentage of circulating hypochromic red blood cells			
CHR - content of haemoglobin in reticulocytes			
TSAT - transferrin saturation			
sTfR - soluble transferrin receptor			

It was found that compared with the control group, RA patients had a higher prevalence of anaemia (5% vs 18%), lower haemoglobin levels, lower haematocrit levels, lower MCV, MCH and MCHC values and higher RDW values. Two RA patients had vitamin B12 deficiency and one RA patient had folic acid deficiency. A higher percentage of RA patients compared with the control group had TSAT values below 20% (43% vs 5%), ferritin levels below the reference range (15% vs 7%), soluble transferrin receptor levels above 1.59 mg/l (26% vs 0%) and hepcidin levels below 14.5 ng/ml (56% vs 2%). Sixty-four per cent of RA patients had iron deficiency (Table 1).

A positive RF was present in 55% of RA patients and anti-CCP antibodies were found in 75% of the patients. Ninety-one per cent of RA patients presented high disease activity, as assessed by DAS-28 (> 5.1). The mean duration of disease in the RA patients studied was 5.5 years. At the time of inclusion in the study, 10% of the patients were taking sulfasalazine at therapeutic doses, 77% were taking methotrexate at therapeutic doses and 42% were taking glucocorticoids at low doses (**Table 2**).

The assessment of correlations between ferritin levels and the parameters studied showed that reduced ferritin levels were observed in younger participants, more frequently in women, participants without hypertension, participants without diabetes, participants with lower triglyceride levels, higher GFR, lower GGT levels, lower uric acid levels and higher platelet counts. No correlations were found between ferritin levels and parameters describing disease activity and the treatment used in RA patients (Table 3). The multi-factor model describing relationships between ferritin levels and the clinical and laboratory parameters in RA patients revealed correlations between reduced ferritin levels and younger age, female gender, lower GGT levels and increased platelet counts (Table 4).

Table 3
Laboratory parameters for RA and treatment used in RA patients

Variable	RA patients, n = 62
RF, [IU/mL]	25.2 (9.75–75.5)
RF > 16 IU/mL, n (%)	32 (55)
Anti-CCP antibodies [IU/mL]	260 (15-5000)
Anti-CCP antibodies > 17 IU/mL, n (%)	44 (75)
DAS-28 [score]	6.5 (6.3–6.8)
DAS-28 > 5.1, n (%)	60 (91)
Period from diagnosis of RA [years]	5.5 (3.0–10.0)
Sulfasalazine, n (%)	6 (10)
Sulfasalazine, daily dose [mg]	2500 (2000–3000)
Methotrexate, n (%)	38 (77)
Methotrexate, weekly dose [mg]	25 (20–25)
Glucocorticoids, n (%)	26 (42)
Glucocorticoids, daily dose[&] [mg]	5 (5.0–7.5)
& Glucocorticoids: dose of steroids expressed as a prednisone-equivalent dose	
RA – rheumatoid arthritis	
RF - rheumatoid factor	
Anti-CCP antibodies - anti-cyclic citrullinated peptide antibodies	
DAS-28 - disease activity score	

Table 4
Correlations between iron metabolism and haemoglobin parameters and clinical and laboratory parameters in RA patients – **single-factor models**

Variables	Basic value	Analysed subgroups	Ferritin [µg/L]	TSAT [%]	Hepcidin [ng/mL]	sTfR [mg/L]	Haemoglobin [g/dL]
Age [years]	r		0.38	0.04	0.38	0.001	-0.03
	p		< 0.01	0.79	< 0.01	1.00	0.79
Sex, male, n (%)	median (Q1 - Q3) or mean ± SD	male	189 (112–364)	30.7 ± 14.5	22.7 (8.1–27.8)	1.43 (1.11–1.61)	14.2 ± 1.9
	median (Q1 - Q3) or mean ± SD	women	74 (38–111)	23.0 ± 13.4	12.9 (6.9–21.0)	1.36 (1.13–1.58)	13.1 ± 1.0
	p		< 0.001	0.11	0.17	0.82	< 0.05
BMI [kg/m²]	r		-0.03	-0.2829	-0.01	0.19	0.08
	p		0.82	0.03	0.97	0.14	0.54
Hypertension, n (%)	median (Q1 - Q3) or mean ± SD	yes	149.0 (86.0–220.5)	24.8 ± 12.7	18.5 (10.6–22.5)	1.41 (1.26–1.81)	13.3 ± 1.1
	median (Q1 - Q3) or mean ± SD	no	72.0 (33.0–105.0)	24.0 ± 14.3	12.7 (4.3–19.9)	1.31 (1.10–1.58)	13.3 ± 1.3
	p		< 0.001	1.0	0.09	0.63	0.91
Diabetes, n (%)	median (Q1 - Q3) or mean ± SD	yes	188.5 (161.5–288.0)	24.0 ± 7.8	17.5 (10.5–24.3)	1.26 (1.04–1.80)	13.2 ± 1.0
	median (Q1 - Q3) or mean ± SD	no	82.0 (41.0–122.0)	24.3 ± 14.1	12.9 (7.1–21.5)	1.36 (1.14–1.61)	13.3 ± 1.3
	p		< 0.05	0.97	0.54	0.89	0.95
Heart failure, n (%)	median (Q1 - Q3) or mean ± SD	yes	100.0 (82.0–364.0)	20.7 ± 10.1	12.8 (6.9–46.7)	1.91 (1.04–2.04)	11.8 ± 0.5
	median (Q1 - Q3) or mean ± SD	no	88.5 (41.0–146.0)	24.5 ± 13.9	13.9 (7.1–21.5)	1.36 (1.13–1.58)	13.3 ± 1.2
	p		0.30	0.65	0.53	0.38	< 0.05
Triglycerides [mg/dL]	r		0.41	0.05	0.27	-0.07	0.30
	p		< 0.01	0.70	< 0.05	0.61	< 0.05
GFR [mL/min/1.73m²]	r		-0.29	-0.02	-0.19	-0.02	-0.06
	p		< 0.05	0.86	0.17	0.90	0.66
Total bilirubin [mg/dL]	r		0.07	0.36	0.01	0.01	0.18
	p		0.59	< 0.01	0.94	0.97	0.16

RA – rheumatoid arthritis

BMI - body mass index

GFR - glomerular filtration rate

AST - aspartate aminotransferase

GGT - gamma-glutamyl transpeptidase

CRP - C-reactive protein

IL-6 - interleukin 6

Anti-CCP antibodies - anti-cyclic citrullinated peptide antibodies

DAS-28 - disease activity score

* in the case of the variable analysed, there were no patients with a given diagnosis or category in the group concerned

Variables	Basic value	Analysed subgroups	Ferritin [µg/L]	TSAT [%]	Hepcidin [ng/mL]	sTfR [mg/L]	Haemoglobin [g/dL]
AST [IU/L]	r		0.14	0.29	0.12	-0.09	0.18
	p		0.29	< 0.05	0.37	0.50	0.15
GGT [IU/L]	r		0.32	0.02	0.18	0.12	0.15
	p		< 0.05	0.86	0.18	0.37	0.25
Uric acid [mg/dL]	r		0.39	0.09	0.34	-0.22	0.32
	p		< 0.01	0.49	< 0.05	0.10	< 0.05
CRP [mg/L]	r		0.15	-0.33	0.30	0.27	-0.28
	p		0.25	< 0.01	< 0.05	< 0.05	< 0.05
IL-6 [pg/mL]	r		0.03	-0.06	0.29	0.19	-0.42
	p		0.80	0.65	< 0.05	0.15	< 0.01
Platelet count [G/L]	r		-0.36	-0.22	-0.05	0.27	-0.27
	p		< 0.01	0.09	0.74	< 0.05	< 0.05
Anti-CCP antibodies [IU/mL]	r		-0.01	0.10	0.28	-0.07	-0.04
	p		0.94	0.45	< 0.05	0.60	0.75
DAS-28 > 5.1, n (%)*	median (Q1 - Q3) or mean ± SD	yes	128 (43–152)	24.5 ± 13.9	13.8 (7.1–21.5)	1.36 (1.11–1.61)	13.3 ± 1.3
	median (Q1 - Q3) or mean ± SD	no	100 (100–100)	16.7 ± 0	47.7 (47.7–47.7)	1.22 (1.22–1.22)	12.5 ± 0
	p		0.85	0.58	< 0.0001	0.85	0.52
RA – rheumatoid arthritis							
BMI - body mass index							
GFR - glomerular filtration rate							
AST - aspartate aminotransferase							
GGT - gamma-glutamyl transpeptidase							
CRP - C-reactive protein							
IL-6 - interleukin 6							
Anti-CCP antibodies - anti-cyclic citrullinated peptide antibodies							
DAS-28 - disease activity score							
* in the case of the variable analysed, there were no patients with a given diagnosis or category in the group concerned							

The assessment of correlations between transferrin saturation and the parameters studied showed that TSAT values were lower in participants with a higher BMI and elevated CRP levels and were higher in participants with elevated bilirubin and AST levels. No correlations were found between TSAT values and parameters describing disease activity and the treatment used in RA patients (Table 3). The multi-factor model describing relationships between TSAT values and the clinical and laboratory parameters in RA patients revealed correlations between reduced TSAT values and reduced haemoglobin levels, lower AST levels as well as higher BMI values and CRP levels (Table 4).

The assessment of correlations between hepcidin levels and the parameters studied showed that lower hepcidin levels were correlated with younger age, lower triglyceride levels, lower uric acid levels and lower CRP and IL-6 levels. A correlation was found between elevated hepcidin levels and parameters describing disease activity (DAS-28 > 5.1 and higher anti-CCP antibody levels) and the treatment used in RA patients. A correlation was also found between elevated hepcidin levels and a higher daily dose of glucocorticoids (Table 3). The multi-factor model describing relationships between hepcidin levels and clinical and laboratory parameters in RA patients revealed correlations between lower hepcidin levels and lower triglyceride levels, lower uric acid levels and lower anti-CCP antibody levels (Table 4).

The analysis of correlations between soluble transferrin receptor levels and the parameters studied showed that higher sTfR levels were correlated with elevated CRP levels, higher platelet counts and the use of glucocorticoids in RA patients (Table 3). The multi-factor model describing relationships between sTfR levels and clinical and laboratory parameters in RA patients revealed correlations between increased sTfR levels and reduced haemoglobin levels (Table 4).

The assessment of correlations between haemoglobin levels and the parameters studied showed that reduced haemoglobin levels were more frequently observed in female participants, patients with heart failure, lower triglyceride levels and lower uric acid levels. Elevated haemoglobin levels were correlated with higher CRP and IL-6 levels and higher platelet counts. No correlations were found between haemoglobin levels and parameters describing disease activity and the treatment used in RA patients (Table 3). The multi-factor model describing relationships between haemoglobin levels and clinical and laboratory parameters in RA patients revealed correlations between reduced haemoglobin levels and reduced uric acid levels, increased IL-6 levels and female gender (Table 4).

In single- and multi-factor models in RA patients with and without iron deficiency, the strongest correlations were found between iron deficiency and younger age, female gender, reduced haemoglobin levels, increased platelet counts, increased GFR, reduced GGT levels, lower disease activity, as assessed by DAS-28, and less frequent use of sulfasalazine (Table 5).

Table 5
Correlations between iron metabolism and haemoglobin parameters and clinical and laboratory parameters in RA patients – **multi-factor models**

Dependent variables	Independent variables	Standardised β	p	Adjusted R^2
Ferritin [1 log µg/L]	Age [years]	0.27	< 0.05	0.41
	Sex [male vs female]	0.37	< 0.01	
	GGT [log IU/L]	0.28	< 0.05	
	Platelet count [G/L]	-0.26	< 0.05	
TSAT [%]	BMI [kg/m²]	-0.27	< 0.05	0.29
	AST [log IU/L]	0.23	< 0.05	
	CRP [mg/L]	-0.24	< 0.05	
	Haemoglobin [g/dL]	0.28	< 0.05	
Hepcidin [1 log ng/dL]	Triglycerides [mg/dL]	0.29	< 0.05	0.32
	Uric acid [mg/dL]	0.33	< 0.05	
	Anti-CCP antibodies [log UI/L]	0.46	< 0.001	
sTfR [1 log mg/L]	Haemoglobin [kg/m^2]	-0.35	< 0.01	0.11
Haemoglobin [g/dL]	Uric acid [mg/dL]	0.26	< 0.05	0.29
	IL-6 [pg/mL]	-0.40	< 0.01	
	Sex [[male vs female]]	-0.25	< 0.05	
TSAT - transferrin saturation				
sTfR - soluble transferrin receptor				
GGT - gamma-glutamyl transpeptidase				
BMI - body mass index				
AST - aspartate aminotransferase				
CRP - C-reactive protein				
Anti-CCP antibodies - anti-cyclic citrullinated peptide antibodies				
IL-6 - interleukin 6				

Table 6
Comparison of the clinical characteristics and basic laboratory parameters in patients with and without iron deficiency and determinants of iron deficiency (**single- and multi-factor models**) in RA patients

Variable	RA patients without ID, n = 22	RA patients with ID, n = 39	p	Category/ unit	Single-factor model				Multi-factor model			
					OR	± 95% CI	χ^2	p	OR	± 95% CI	χ^2	p
Age [years]	57 ± 14	49 ± 16	0.05	5 years	0.83	0.69–1.01	3.54	0.06	0.95	0.91–1.00	4.31	0.04
Sex, male, n (%)	10 (45)	0	< 0.001	-£	-	-	-	-	-	-	-	-
GFR [mL/min/1.73 m^2]	89 ± 19	99 ± 18	< 0.05	10 mL/min/1.73 m^2	1.4	1.01–1.93	4.27	0.04	-	-	-	-
GGT [IU/L]	26 (21–43)	20 (18–29)	< 0.05	1 log IU/L	0.31	0.09–1.04	3.74	0.05	-	-	-	-
Platelet count [G/L]	259 ± 67	316 ± 96	< 0.05	10 g/L	1.12	1.02–1.23	5.47	0.02	-	-	-	-
Haemoglobin [g/dL]	13.7 ± 1.5	13.0 ± 1.0	< 0.05	g/dL	0.63	0.39–1.01	3.83	0.05	0.54	0.31–0.95	4.82	0.03
DAS-28 [score]	6.7 (6.5–6.8)	6.5 (5.8–6.8)	< 0.05	1 point	0.22	0.06–0.84	5.08	0.03	0.25	0.06–0.96	4.23	0.04
Sulfasalazine, n (%)	5 (23)	1 (3)	< 0.05	yes vs no	0.09	0.01–0.86	4.53	0.03	-	-	-	-

£ - it is impossible to build a logistic model due to 0 or 1 in one of the categories analysed

RA – rheumatoid arthritis

ID - iron deficiency

GFR - glomerular filtration rate

GGT - gamma-glutamyl transpeptidase

DAS-28 - disease activity score

Discussion

Surprisingly, despite the pathophysiological relationship between iron deficiency and anaemia as well as inflammation, data on iron metabolism in RA patients are quite enigmatic. The studies available focus only on anaemia in the context of iron deficiency in RA patients [20, 21] and do not take into account new, more specific, biomarkers (e.g. soluble transferrin receptor, hepcidin) in the assessment of iron metabolism [4, 5, 6]. It is not clear, either, whether anti-inflammatory treatment (e.g. blocking the IL-6 pathway) may, at least partly, normalise iron levels in RA patients.

It was demonstrated that iron deficiency, defined on the basis of serum ferritin levels and transferrin saturation, is very common in RA patients both with and without anaemia. It was also found that RA patients with iron deficiency have very low serum hepcidin levels, which indicates that those patients have predominantly absolute rather than, as one would expect, functional (relative) iron deficiency.

To date, iron metabolism in RA patients has generally been analysed in the literature only in the context of anaemia [2, 10, 17, 18, 22, 23]. Anaemia is commonly reported in RA patients and its prevalence, according to various publications, is 33–60% [2, 10]. The present study demonstrated that only 18% of the RA patients analysed had anaemia, whereas the prevalence of iron deficiency in those patients was over three times higher (64%). The pathomechanism of anaemia in RA patients is complex and multifactorial and is linked to chronic inflammation and the deficiency/loss of particular components of erythropoiesis, including iron deficiency [10, 24]. It was shown that anaemia in RA patients leads to a more severe course of the underlying condition and more advanced damage to the joint structure [2, 23, 25].

Recently, a hypothesis has been proposed that the main factor leading to the development of anaemia in the course of chronic conditions (e.g. chronic kidney disease, RA) is excessive inflammatory activation, linked, among other things, to the production of proinflammatory cytokines, especially IL-6, which leads to an increase in the production of hepcidin in the liver [14, 26, 27]. Models of chronic conditions, in which the activation of inflammation is an important pathophysiological feature, have provided data suggesting that IL-6-mediated overproduction of

hepcidin in the liver leads to the development of the so-called functional (relative) iron deficiency (there are iron stores in the body, but the element is unavailable for cell metabolic processes) [4, 28, 29, 30]. This phenomenon was reported in patients with chronic kidney disease; it was observed that patients with chronic kidney disease and iron deficiency have very high serum hepcidin levels [31]. It should be stressed that in the case of RA, these are only theoretical assumptions, as it is yet unclear to what extent the excessive inflammatory activation (linked to overexpression of IL-6 and hepcidin) observed in RA patients leads to functional (relative) iron deficiency and to what extent iron deficiency in RA patients constitutes absolute iron deficiency (as may be indicated by reduced serum hepcidin levels). It should be noted that while it has theoretically been suggested that iron deficiency in patients with heart failure is primarily functional (relative) iron deficiency, this has not yet been confirmed. However, there is data indicating that patients with iron deficiency have reduced (rather than increased) serum hepcidin levels. This was observed in patients with stable heart failure as well as those with acute heart failure. Also, there is no correlation between increased IL-6 levels and increased serum hepcidin levels in this group of patients [32, 33].

In the RA patients studied, iron metabolism was analysed using classic parameters that have been in use for many years now (serum ferritin levels and transferrin saturation) and new biomarkers (serum soluble transferrin receptor and hepcidin levels) [22, 34, 35]. There are numerous, different definitions of iron deficiency in the literature. Given practical implications (relating to e.g. the qualification of patients for iron supplementation treatment), the definition of iron deficiency used in the case of RA patients participating in this study was the one that is used in patients with chronic conditions, such as heart failure and chronic kidney disease [6]. Iron deficiency was found in 64% of RA patients, including in 76% of women. Thus, iron deficiency was common among RA patients and its prevalence was several times higher than the prevalence of anaemia. It needs to be stressed that the prevalence of iron deficiency in RA patients with anaemia was similar to the prevalence of iron deficiency in RA patients without anaemia (66% and 55%, respectively). The study clearly indicates that the scale of the iron deficiency problem is significantly bigger as compared to anaemia. Therefore, it is surprising that iron deficiency has, until now, not been taken into account in the clinical assessment of RA patients.

However, it is obvious that iron metabolism is associated with the effectiveness of erythropoiesis in patients with chronic conditions, too [36]. In the present study, correlations were found between reduced haemoglobin levels as well as reduced red blood cell parameters (MCV, MCH, CHR) and parameters describing iron metabolism (reduced serum ferritin and hepcidin levels, increased serum sTfR levels and reduced transferrin saturation) in the RA patients studied. In the multi-factor model, reduced haemoglobin levels and increased DAS-28 scores were the two strongest determinants of iron deficiency in RA patients. Our study shows that, as might have been expected, there is a correlation between iron metabolism and erythropoiesis in RA patients. However, iron deficiency is also common in RA patients displaying parameters indicative of normal erythropoiesis. This clinically important observation proves that normal haemoglobin levels do not exclude iron deficiency. Thus, in order to diagnose iron deficiency in a rheumatoid arthritis patient, iron metabolism parameters should be assessed in addition to haemoglobin levels and other blood cell parameters.

In the present study, RA patients had lower serum ferritin levels compared with healthy controls. This was the case even though inflammatory activation in RA patients was higher compared with healthy controls, which may indirectly indicate the presence of a large absolute iron deficiency, independent of a concurrent inflammatory process. Similar differences were also observed as regards serum hepcidin levels. RA patients, regardless of the presence of iron deficiency, had lower serum hepcidin levels compared with healthy controls. Moreover, RA patients with iron deficiency had lower serum hepcidin levels compared with RA patients without iron deficiency. No clear correlations were found between iron metabolism parameters and inflammation parameters (CRP, IL-6) in RA patients. The above data do not confirm that there is a link between inflammatory activation and the pathogenesis of iron deficiency in RA patients, or at least it appears that inflammatory activation does not play a dominant role in the pathogenesis of iron deficiency in RA patients.

Conclusions

1. Iron deficiency is common in RA patients with high disease activity (DAS-28 > 5.1). Its prevalence in these patients is 64%. Iron deficiency is comparably common in RA patients with anaemia and those without anaemia (66% vs 55%).

2. Compared with healthy controls, RA patients had lower transferrin saturation, lower ferritin and hepcidin levels and higher serum sTfR levels. RA patients with iron deficiency had lower serum hepcidin levels compared with RA patients without iron deficiency.

3. In the case of RA patients, correlations were found between reduced haemoglobin levels as well as reduced red blood cell parameters (MCV, MCH, CHR) and parameters describing iron metabolism (reduced serum ferritin and hepcidin levels, increased serum sTfR levels and reduced transferrin saturation).

4. No correlations were found in single-factor models between iron deficiency and inflammatory markers (IL-6, CRP) as well as parameters describing the disease (RF, anti-CCP antibodies) in RA patients. In multi-factor models, increased DAS-28 scores and reduced haemoglobin levels were the two strongest determinants of iron deficiency in RA patients.

Declarations

1. Ethics approval and consent to participate.

The study was approved by the Commission of Bioethics at Military Institute of Medicine in Warsaw (No 34/WIM/2015).

2. Consent for publication.

Written informed consent in Polish was obtained from the all the patients for the publication of this paper.

3. Competing interests.

The authors declare that they have no competing interests.

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5. Acknowledgements.

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6. Author contributions.

WT wrote the manuscript. MC helped to draft the manuscript. BJP participated in the design of the study and arranged the manuscript. EAJ participated in the design and coordination of the paper. All the authors read and approved the final manuscript.

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